

COURTESY COPY OF PENDING CLAIMS IN USSN 09/932,254
(as amended on March 5, 2002)

1. A method of producing a modified gene fusion construct, the method comprising cojoining two or more heterologous nucleic acid sequences, wherein each heterologous nucleic acid sequence encodes one or more enzymatic domains, and wherein at least one of the two or more heterologous nucleic acid sequences is modified, thereby producing the modified gene fusion construct.
2. The method of claim 1, wherein at least one of the two or more heterologous nucleic acid sequences is modified prior to cojoining the two or more heterologous nucleic acid sequences.
3. The method of claim 1, wherein at least one of the two or more heterologous nucleic acid sequences is modified after cojoining the two or more heterologous nucleic acid sequences.
4. The method of claim 1, wherein the one or more enzymatic domains participate in a same metabolic pathway.
5. The method of claim 1, wherein at least one of the two or more heterologous nucleic acid sequences is modified by shuffling at least one nucleic acid sequence.
6. The method of claim 5, wherein shuffling the at least one nucleic acid sequence comprises recursive sequence recombination.
7. The method of claim 1, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of phytoene synthase, phytoene desaturase, z-carotene desaturase, and beta-cyclase.
8. The method of claim 1, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of diaminobutyric acid aminotransferase, diaminobutyric acid acetyltransferase, and ectoine synthase.
9. The method of claim 1, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of beta-ketothiolase, D-reductase, and poly(hydroxyalkanoate) synthase.

10. The method of claim 1, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of a ketosynthase-acyltransferase, a chain length factor, an acyl carrier protein, and a cyclase.

11. The method of claim 1, wherein cojoining the two or more heterologous nucleic acid sequences comprises connecting the two or more heterologous nucleic acid sequences directly to one another.

12. The method of claim 1, wherein cojoining the two or more heterologous nucleic acid sequences comprises connecting the two or more heterologous nucleic acid sequences with one or more nucleotide linker sequences.

13. The method of claim 12, wherein the one or more nucleotide linker sequences independently comprise between about three and about 300 nucleotides.

14. The method of claim 13, wherein the one or more nucleotide linker sequences independently comprise between about 12 to about 90 nucleotides.

15. The method of claim 12, wherein at least one of the one or more nucleotide linker sequences comprises one or more intron sequences.

16. The method of claim 12, wherein at least one of the one or more nucleotide linker sequences comprises a nucleotide sequence that encodes a peptide linker.

17. The method of claim 16, wherein the peptide linker comprises a cleavable peptide sequence or an intein sequence.

18. The method of claim 16, wherein at least about 80% of the amino acid residues in the peptide linker are selected from the group consisting of alanine and glycine residues.

19. The method of claim 1, wherein the modified gene fusion construct further comprises one or more transcription regulatory sequences.

20. The method of claim 19, wherein the one or more transcription regulatory sequences comprises one or more plant transcription regulatory sequences.

21. The method of claim 1, further comprising introducing the modified gene fusion construct into a eukaryotic system.

22. The method of claim 21, wherein the eukaryotic system is a plant system.

23. The method of claim 22, wherein the plant system is selected from the group consisting of *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, *Malus*, *Apium*, *Narcissus*, *Docus*, and *Datura*.

24. A transgenic plant prepared by the method of claim 22.

25. A modified gene fusion construct comprising two or more cojoined heterologous nucleic acid sequences, wherein each nucleic acid sequence encodes one or more enzymatic domains, and wherein at least one of the two or more cojoined heterologous nucleic acid sequences is modified.

26. The modified gene construct of claim 25, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is shuffled.

27. The modified gene fusion construct of claim 25, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of phytoene synthase, phytoene desaturase, z-carotene desaturase, and beta-cyclase.

28. The modified gene fusion construct of claim 25, wherein the two or more cojoined nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of diaminobutyric acid aminotransferase, diaminobutyric acid acetyltransferase, and ectoine synthase.

29. The modified gene fusion construct of claim 25, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of beta-ketothiolase, D-reductase, and poly(hydroxyalkanoate) synthase.

30. The modified gene fusion construct of claim 25, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of a ketosynthase-acyltransferase, a chain length factor, an acyl carrier protein, and a cyclase.

31. A vector comprising the modified gene fusion construct of claim 25 and a promoter.

32. A method of producing a gene fusion construct, the method comprising cojoining two or more heterologous nucleic acid sequences that participate in the same metabolic pathway, wherein at least one of the cojoined nucleic acid sequences is derived from a eukaryote and another cojoined nucleic acid sequence is derived from either a different species of eukaryote or from a prokaryote.

33. The method of claim 32, wherein at least one of the cojoined nucleic acid sequences is derived from a plant.

34. The method of claim 32, wherein at least one of the cojoined nucleic acid sequences is derived from a prokaryote.

35. The method of claim 34, wherein at least one of the cojoined nucleic acid sequences is derived from a plant.

36. The method of claim 32, wherein at least two of the cojoined nucleic acid sequences are derived from different plant species.

37. The method of claim 32, wherein the method comprises cojoining three or more heterologous nucleic acid sequences that participate in the same metabolic pathway.

38. The method of claim 32, wherein at least one of the cojoined heterologous nucleic acid sequences is modified.

39. The method of claim 38, wherein at least one of the cojoined heterologous nucleic acid sequences is shuffled.

40. The method of claim 32, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of phytoene synthase, phytoene desaturase, α -carotene desaturase, and beta-cyclase.

41. The method of claim 32, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of diaminobutyric acid aminotransferase, diaminobutyric acid acetyltransferase, and ectoine synthase.

42. The method of claim 32, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of beta-ketothiolase, D-reductase, and poly(hydroxyalkanoate) synthase.

43. The method of claim 32, wherein the two or more heterologous nucleic acid sequences encode enzymatic domains selected from the group consisting of a ketosynthase-acyltransferase, a chain length factor, an acyl carrier protein, and a cyclase.

44. The method of claim 32, wherein the cojoined nucleic acid sequences are connected directly to one another.

45. The method of claim 32, wherein the cojoined nucleic acid sequences are connected to one another with one or more nucleotide linker sequences.

46. The method of claim 32, wherein the modified gene fusion construct further comprises one or more transcription regulatory sequences.

47. The method of claim 46, wherein the one or more transcription regulatory sequences comprises one or more plant transcription regulatory sequences.

48. The method of claim 32, further comprising introducing the modified gene fusion construct into a eukaryotic system.

49. The method of claim 48, wherein the eukaryotic system is a plant system.

50. The method of claim 49, wherein the plant system is selected from the group consisting of *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, *Malus*, *Apium*, *Narcissus*, *Docus*, and *Datura*.

51. A transgenic plant prepared by the method of claim 49.

52. A gene fusion construct comprising two or more cojoined heterologous nucleic acid sequences that participate in the same metabolic pathway, wherein at least one of the cojoined heterologous nucleic acid sequences is derived from a eukaryote and another cojoined heterologous nucleic acid sequence is derived from either a different species of eukaryote or from a prokaryote.

53. The gene fusion construct of claim 52, wherein at least one of the cojoined heterologous nucleic acid sequences is modified.

54. The gene fusion construct of claim 53, wherein at least one of the cojoined heterologous nucleic acid sequences is shuffled.

55. The gene fusion construct of claim 52, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of phytoene synthase, phytoene desaturase, z-carotene desaturase, and beta-cyclase.

56. The gene fusion construct of claim 52, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of diaminobutyric acid aminotransferase, diaminobutyric acid acetyltransferase, and ectoine synthase.

57. The gene fusion construct of claim 52, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of beta-ketothiolase, D-reductase, and poly(hydroxyalkanoate) synthase.

58. The gene fusion construct of claim 52, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of a ketosynthase-acyltransferase, a chain length factor, an acyl carrier protein, and a cyclase.

59. A vector comprising the gene fusion construct of claim 52 and a promoter.

60. A gene fusion construct comprising two or more cojoined heterologous nucleic acid sequences, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of diaminobutyric acid aminotransferase, diaminobutyric acid acetyltransferase, and ectoine synthase.

61. The gene fusion construct of claim 60, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is modified.

62. The gene fusion construct of claim 61, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is shuffled.

63. A gene fusion construct comprising three or more cojoined heterologous nucleic acid sequences, wherein the three or more cojoined heterologous nucleic acid sequences encode at least three enzymatic domains selected from the group consisting of beta-ketothiolase, D-reductase, and poly(hydroxyalkanoate) synthase.

64. The gene fusion construct of claim 63, wherein at least one of the three or more cojoined heterologous nucleic acid sequences is modified.

65. The gene fusion construct of claim 64, wherein at least one of the three or more cojoined heterologous nucleic acid sequences is shuffled.

66. A gene fusion construct comprising two or more cojoined heterologous nucleic acid sequences, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of a ketosynthase-acyltransferase, a chain length factor, an acyl carrier protein, and a cyclase.

67. The gene fusion construct of claim 66, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is modified.

68. The gene fusion construct of claim 67, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is shuffled.

69. A gene fusion construct comprising two or more cojoined heterologous nucleic acid sequences, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of a phytoene synthase, phytoene desaturase, z-carotene desaturase, and beta-cyclase.

70. The gene fusion construct of claim 69, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is modified.

71. The gene fusion construct of claim 70, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is shuffled.

72. A hybrid protein comprising two or more heterologous enzymatic domains that participate in the same metabolic pathway, wherein at least one of the two or more heterologous enzymatic domains is encoded by a nucleic acid sequence that has been modified.

73. The hybrid protein of claim 72, wherein at least one of the two or more heterologous enzymatic domains is encoded by a nucleic acid sequence that has been shuffled.

74. The hybrid protein of claim 72, wherein two or more heterologous enzymatic domains are connected by one or more peptide linker sequences.

75. The hybrid protein of claim 74, wherein at least one of the one or more peptide linker sequences comprises a cleavable peptide sequence or an intein sequence.

76. The hybrid protein of claim 72, wherein the heterologous enzymatic domains that participate in the same metabolic pathway comprise one or more of phytoene synthase, phytoene desaturase, z-carotene desaturase, or beta-cyclase.

77. The hybrid protein of claim 72, wherein the heterologous enzymatic domains that participate in the same metabolic pathway comprise one or more of diaminobutyric acid aminotransferase, diaminobutyric acid acetyltransferase, or ectoine synthase.

78. The hybrid protein of claim 72, wherein the heterologous enzymatic domains that participate in the same metabolic pathway comprise one or more of beta-ketothiolase, D-reductase, or poly(hydroxyalkanoate) synthase.

79. The hybrid protein of claim 72, wherein the heterologous enzymatic domains that participate in the same metabolic pathway comprise one or more of a ketosynthase-acyltransferase, a chain length factor, an acyl carrier protein, or a cyclase.

80. A method of producing a gene fusion construct, the method comprising cojoining two or more nucleic acid sequences encoding at least two enzymatic domains, wherein at least one of the nucleic acid is derived from a plant, thereby producing a gene fusion construct.

81. The method of claim 80, wherein the at least two enzymatic domains comprise domains from plant enzymes selected from the group consisting of phytoene synthase, phytoene desaturase, z-carotene desaturase, and beta-cyclase.

82. The method of claim 80, wherein the at least two enzymatic domains are derived from proteins that participate in the same metabolic pathway.

83. The method of claim 80, wherein at least one of the two or more nucleic acid sequences is modified.

84. The method of claim 83, wherein at least one of the two or more nucleic acid sequences is modified by shuffling.

85. The method of claim 84, wherein shuffling the at least one nucleic acid sequence comprises recursive sequence recombination.

86. The method of claim 80, wherein cojoining the two or more nucleic acid sequences comprises connecting the two or more nucleic acid sequences directly to one another.

87. The method of claim 80, wherein cojoining the two or more nucleic acid sequences comprises connecting the two or more nucleic acid sequences with one or more nucleotide linker sequences.

88. The method of claim 87, wherein the one or more nucleotide linker sequences independently comprise between about three and about 300 nucleotides.

89. The method of claim 88, wherein the one or more nucleotide linker sequences independently comprise between about 12 to about 90 nucleotides.

90. The method of claim 87, wherein at least one of the one or more nucleotide linker sequences comprises one or more intron sequences.

91. The method of claim 87, wherein at least one of the one or more nucleotide linker sequences comprises a nucleotide sequence that encodes a peptide linker.

92. The method of claim 91, wherein the peptide linker comprises a cleavable peptide sequence or an intein sequence.

93. The method of claim 91, wherein at least about 80% of amino acid residues in the peptide linker are selected from the group consisting of alanine and glycine residues.

94. The method of claim 80, wherein the gene fusion construct further comprises one or more transcription regulatory sequences.

95. The method of claim 94, wherein the one or more transcription regulatory sequences comprises one or more plant transcription regulatory sequences.

96. The method of claim 80, further comprising introducing the gene fusion construct into a eukaryotic system.

97. The method of claim 96, wherein the eukaryotic system is a plant system.

98. The method of claim 97, wherein the plant system is selected from the group consisting of *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, *Malus*, *Apium*, *Narcissus*, *Docus*, and *Datura*.

99. A transgenic plant prepared by the method of claim 97.

100. A gene fusion construct comprising two or more cojoined heterologous nucleic acid sequences, wherein each nucleic acid sequence encodes one or more enzymatic domains, and wherein at least one of the two or more cojoined heterologous nucleic acid sequences is derived from a plant.

101. The gene fusion construct of claim 100, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is modified.

102. The gene fusion construct of claim 101, wherein at least one of the two or more cojoined heterologous nucleic acid sequences is shuffled.

103. The gene fusion construct of claim 100, wherein the two or more cojoined heterologous nucleic acid sequences encode at least two enzymatic domains selected from the group consisting of phytoene synthase, phytoene desaturase, z-carotene desaturase, and beta-cyclase.

104. A vector comprising the gene fusion construct of claim 100 and a promoter.